

REMARKS

The above amendments to the specification have been made to incorporate cross-reference to related applications and to be in compliance with CFR §1.821-1.825. Accordingly, Applicant believes no new matter is added by these amendments.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

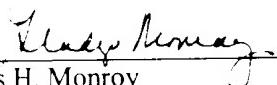
In the unlikely event that the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 514012000300.

The Assistant Commissioner is NOT authorized to charge the cost of the issue fee, extra claim fees or filing fees to the Deposit Account. Applicant wishes to pay extra claim and filing fees with Response to Missing Parts of Application.

Respectfully submitted,

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By:

  
Gladys H. Monroy  
Registration No. 32,430

Morrison & Foerster LLP  
755 Page Mill Road  
Palo Alto, California 94304-1018  
Telephone: (650) 813-5711  
Facsimile: (650) 494-0792

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

*Please replace the following paragraph starting on page 18, line 18 and ending on page 19, line 3*

*(TWICE AMENDED)*

Fig. 2 (SEQ ID NOS:2 and 3) shows a schematic diagram illustrating the test constructs generated, in which stable stem-loop structures were inserted into the Nco I site of the WT1 gene. A Sau 3AI fragment of the WT1 gene was inserted into pSP65(T), positioning a tract of 38 adenine residues downstream of the WT1 gene, allows first strand synthesis to be primed by an oligo d(T) primer. Clones containing either one or two copies of the (M1/X) stem-loop structures were isolated and characterized by sequencing. Insertion of one or two copies of GNRA stem-loop structure was done in f1WT1, a derivative of pSP/WT1 in which a portion of the GC-rich 5' UTR of WT1 was present. Termination of RT by the stem-loop structures is expected to generate a truncated product of ~920 bases, whereas full-length copying of the template is expected to produce a product of ~1.4 - 1.5 kb in the case of pSP/WT1(M1/X) and a product of ~1.9 - 2.0 kb in the case of pSP/f1WT1(GNRA).

*Please replace the following paragraph starting on page 21, line 2 and ending on line 22*

The procedure for generating cDNA libraries has not extensively deviated from the original method of Gubler and Hoffmann (4). A major limitation of the current technology is that a set of products of variable length are often generated during first strand synthesis. Consequently, a number of truncated clones will be present in libraries for any given gene. The difficulty which RT has in transcribing GC-rich regions is well documented. In fact, there are specific RNA structures, called CUUCGG hairpins, which form extraordinarily stable RNA secondary structures capable of blocking RT processivity (2). We have engineered two types of stable stem-loop structures into an Nco I site positioned 918 bp upstream of the Wilm's Tumor WT1 tumor suppressor 3' end (Fig. 2 (SEQ ID NOS:2 and 3)). Plasmid SP/f1WT1 contains 433 bp of the 5' untranslated region of WT1 and is ~ 70% GC rich. Indeed, when cDNA clones for the murine WT1 gene were first isolated, none of the clones were full-length and five of nine clones terminated within 21 nucleotides of each other 182 bases upstream of the ATG codon, suggesting the presence of a strong RT stop signal in this region. The murine WT1 5' end could only be obtained by genomic DNA sequencing (Pelletier et al., 1991, Genes Dev. 5, 1345-1356). We have used in vitro generated WT1 transcripts (ranging in size from ~1.4 - 2.0 kb) to elucidate and optimize conditions which are most effective in allowing RTs of various sources to proceed through these processivity blocks.